

Gap sampling of phenotypic Spotted Bass and Guadalupe Bass from the Little River Watershed

Samples of phenotypic *Micropterus treculii* (GB: Guadalupe Bass; n = 93), *M. punctulatus* (SP: Spotted Bass; n = 19), and *M. salmoides* (LM: Largemouth Bass; n = 98) were collected from the Little River watershed as part of the Texas Natural History Collection's (TNHC) gap sampling effort. Tissue from these samples were lent to the Analytical Services Laboratory for analysis.

For all GB and SP, isolated DNA was genotyped at 16 species informative SNPs (assays BBID1 and BBID2). For a subset of LM (n = 16) the same assays were applied. Multilocus genotypes were then used to assign taxonomic status based on manual inspection, for diagnostic alleles, and using the Bayesian inference algorithm implemented within STRUCTURE (Pritchard et al. 2000; burn-in 20000, mcmc 250000, admix model, allele freq corr, update allele freq w pop flag = 1, K = 4, 20 unknowns per run).

Results based on manual inspection and Bayesian inference were congruent among all samples sans four individuals where the estimated hybrid type differed.

All phenotypic LM were genetically consistent with LM and were not evaluated further here.

Most phenotypic SP (17 of 19) were genetically consistent with SP x GB hybrids, except for two individuals identified as non-introgressed SP (Appendix 1). A majority of these hybrids (15 of 17) were inferred to have greater genetic influence from the SP lineage than from GB (mean SP influence in hybrids: 63%; Figure 1). Both non-introgressed SP were recovered from the same site (Figure 2).

Most phenotypic GB (62 of 93) were genetically consistent with SP x GB hybrids. A majority of these hybrids (55 of 62) were inferred to have greater genetic influence from the GB lineage than from SP (mean GB influence in hybrids: 64%; Figure 1). Other hybrid types were also resolved including GB x SM (*M. dolomieu* – Smallmouth Bass) (5 of 93; mean GB influence 86%) and tribrids (SP x GB x SM; 5 of 93; mean GB influence 46%, mean SP influence 36%, mean SM influence 17%). Non-introgressed taxa were also recovered including GB (18 of 93) and LM (3 of 93). A majority of non-introgressed GB (12 of 18) were recovered from a single site (Brushy Creek) (Figure 2).

Conclusion

Outside of phenotypic LM most fish collected were hybrids. Samples identified to species by phenotype were typically hybrids of the described taxon with a majority of their genome derived from the lineage of the described taxon. Results support previous work indicating that non-introgressed GB could be consistently recovered from Brushy Creek but that GB were not consistently located in surrounding drainages (TPWD, unpublished data). Results are also consistent with previous work (Lutz-Carrillo et al. 2018, Transactions of the American Fisheries Society) suggesting that the Brazos River drainage was part of a natural hybrid zone between Spotted Bass and Guadalupe Bass.

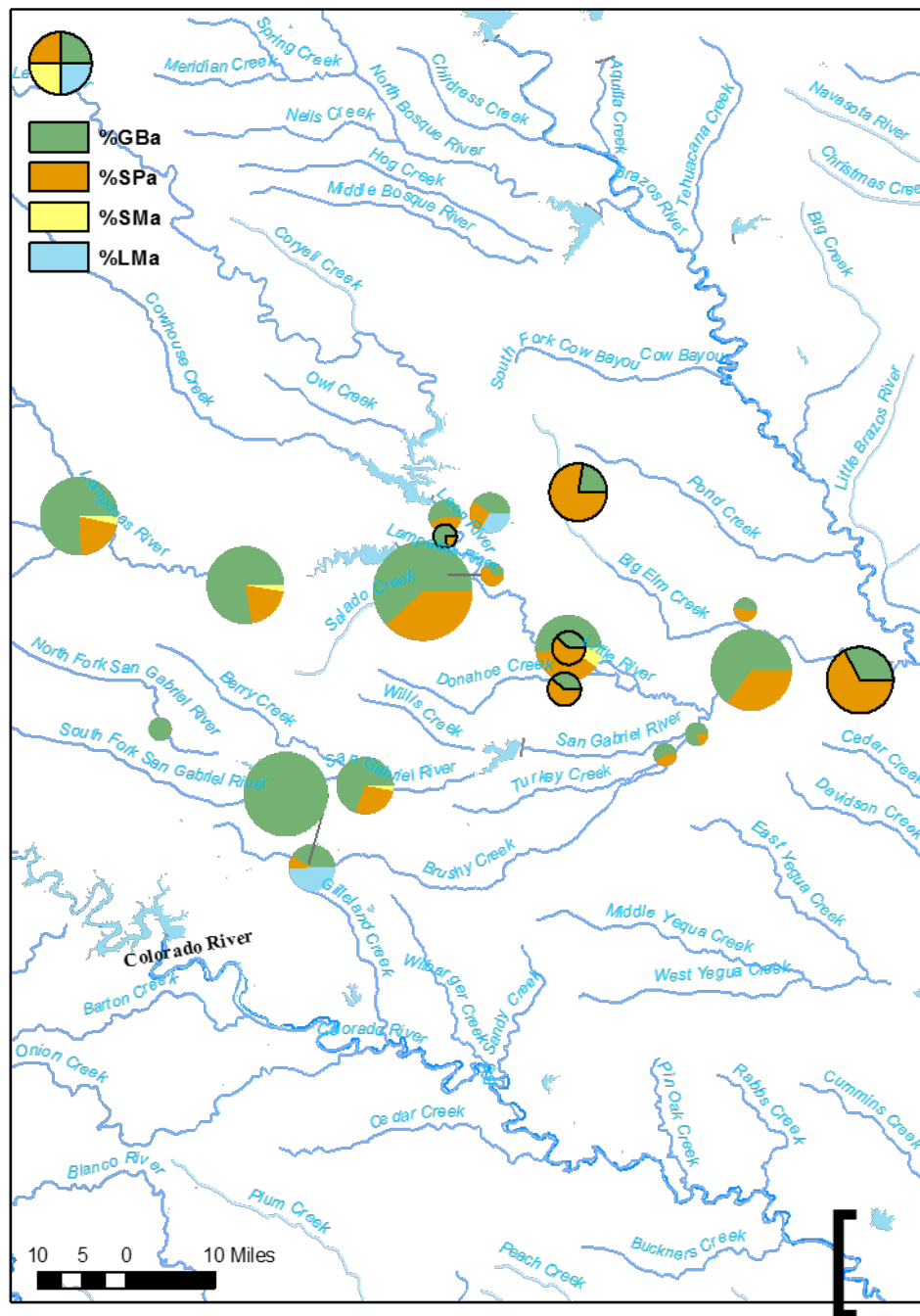


Figure 1. Allele frequencies in collections of phenotypic GB (pie graphs) and phenotypic SB (outlined pie graphs) from the Little River watershed. Graphs are scaled by sample size.

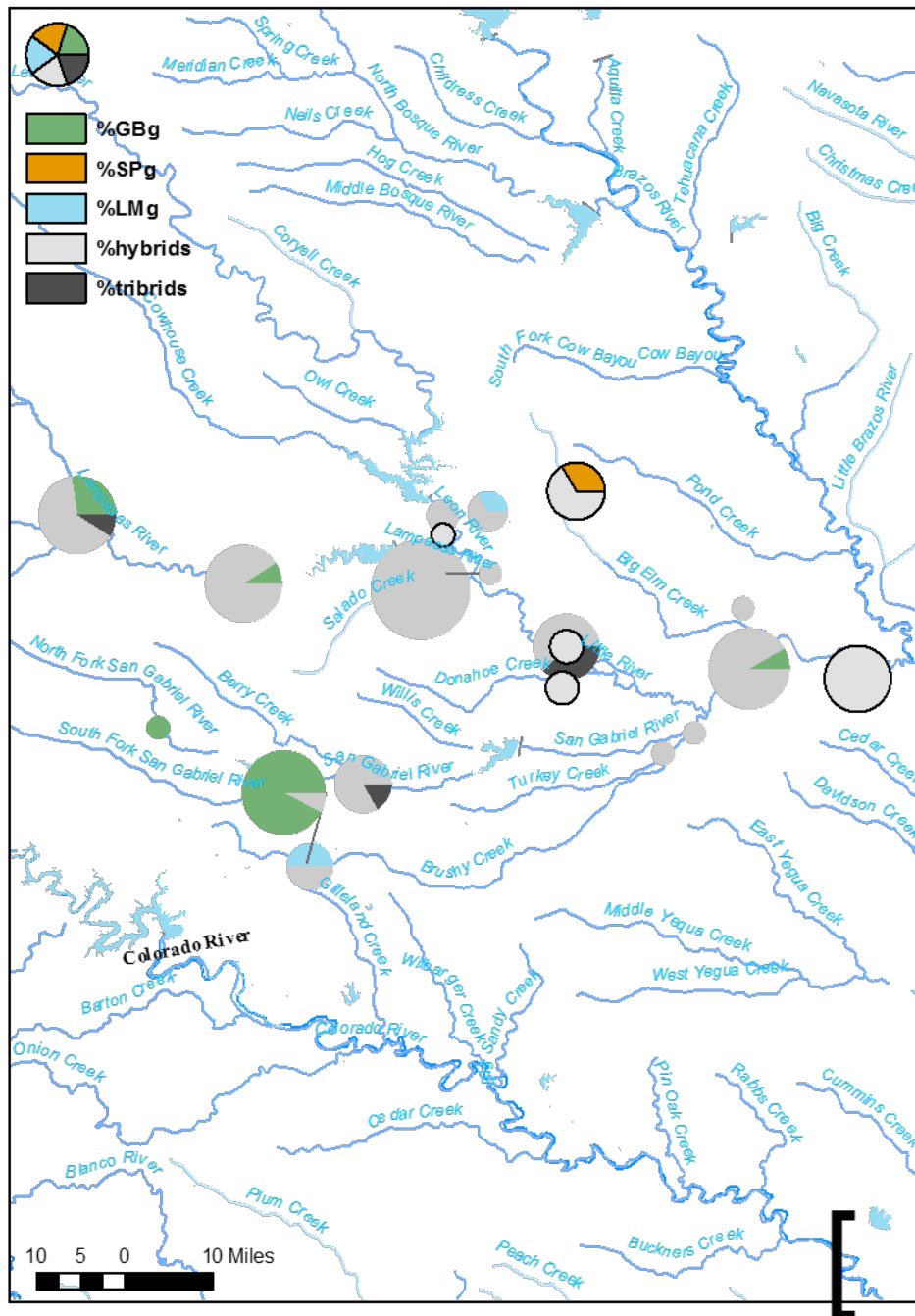


Figure 2. Genotype frequencies in collections of phenotypic GB (pie graphs) and phenotypic SB (outlined pie graphs) from the Little River watershed. Graphs are scaled by sample size.

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Appendix 1. Estimated genotype (gID), and taxa specific allele frequency estimates (GB, SP, SM, LM), for all evaluated samples of phenotypic (Description) GB, SP, and LM from the Little River watershed. Lab ID is equivalent to the submitted TNHC-FT#.

Lab ID	Description	gID	GB	SP	SM	LM	Locality name
TNH-1683	punctulatus	SP x GB	0.33	0.67	0.00	0.00	Donahoe Creek at FM437
TNH-1684	punctulatus	SP x GB	0.45	0.54	0.00	0.00	Donahoe Creek at FM437
TNH-1703	punctulatus	SP x GB	0.39	0.61	0.00	0.00	Little River @ FM437 (~300m reach running above and below bridge)
TNH-1704	punctulatus	SP x GB	0.37	0.62	0.00	0.00	Little River @ FM437 (~300m reach running above and below bridge)
TNH-1711	punctulatus	SP x GB	0.09	0.89	0.01	0.01	Big Elm Creek at FM438
TNH-1712	punctulatus	SP x GB	0.28	0.71	0.00	0.00	Big Elm Creek at FM438
TNH-1713	punctulatus	SP	0.01	0.99	0.00	0.00	Big Elm Creek at FM438
TNH-1714	punctulatus	SP x GB	0.65	0.34	0.00	0.00	Big Elm Creek at FM438
TNH-1715	punctulatus	SP x GB	0.28	0.71	0.00	0.00	Big Elm Creek at FM438
TNH-1716	punctulatus	SP	0.02	0.98	0.00	0.00	Big Elm Creek at FM438
TNH-1729	punctulatus	SP x GB	0.15	0.85	0.00	0.00	Pin Oak Creek at FM2095
TNH-1730	punctulatus	SP x GB	0.25	0.74	0.00	0.00	Pin Oak Creek at FM2095
TNH-1731	punctulatus	SP x GB	0.28	0.71	0.00	0.00	Pin Oak Creek at FM2095
TNH-1732	punctulatus	SP x GB	0.35	0.64	0.01	0.00	Pin Oak Creek at FM2095
TNH-1733	punctulatus	SP x GB	0.43	0.57	0.01	0.00	Pin Oak Creek at FM2095
TNH-1734	punctulatus	SP x GB	0.39	0.61	0.00	0.00	Pin Oak Creek at FM2095
TNH-1735	punctulatus	SP x GB	0.39	0.61	0.00	0.00	Pin Oak Creek at FM2095
TNH-1737	punctulatus	SP x GB	0.37	0.63	0.00	0.01	Pin Oak Creek at FM2095
TNH-1793	punctulatus	SP x GB	0.77	0.22	0.00	0.00	Nolan Creek at E Ave A
TNH-1556	salmoides	LM	0.00	0.00	0.00	1.00	North Fork Leon River at SH112 (~100m reach)
TNH-1576	salmoides	LM	0.00	0.00	0.00	1.00	Sabana River at CR435
TNH-1687	salmoides	LM	0.00	0.00	0.00	1.00	Donahoe Creek at FM437
TNH-1728	salmoides	LM	0.00	0.00	0.00	1.00	Pin Oak Creek at FM2095
TNH-1744	salmoides	LM	0.00	0.00	0.00	1.00	Buttermilk Creek at Gault Site
TNH-1748	salmoides	LM	0.00	0.00	0.00	1.00	Bear Creek on Collins Ranch
TNH-1783	salmoides	LM	0.00	0.00	0.00	1.00	Knob Creek at Reed Cemetery Rd.
TNH-1798	salmoides	LM	0.00	0.00	0.00	1.00	South Nolan Creek at Backstroms Crossing
TNH-1817	salmoides	LM	0.00	0.00	0.00	1.00	Salado Creek at E. Amity Rd
TNH-1854	salmoides	LM	0.00	0.00	0.00	1.00	Oatmeal Creek at FM1174
TNH-1861	salmoides	LM	0.00	0.00	0.00	1.00	Little Rocky Creek at US183 (into S. Rocky Creek)
TNH-1866	salmoides	LM	0.00	0.00	0.00	1.00	Sulphur Creek at FM1715
TNH-1887	salmoides	LM	0.00	0.00	0.00	1.00	Simms Creek at US281
TNH-1890	salmoides	LM	0.00	0.00	0.00	1.00	Cowhouse Creek at CR505 (Schoolerville Rd)
TNH-1895	salmoides	LM	0.00	0.00	0.00	1.00	Cowhouse Creek at CR137
TNH-1896	salmoides	LM	0.00	0.00	0.00	1.00	Coryell Creek at FM107
TNH-1698	treculii	GB x SP	0.48	0.52	0.00	0.00	Big Elm Creek at CR240
TNH-1702	treculii	GB x SP x SM	0.55	0.32	0.13	0.00	Little River @ FM437 (~300m reach running above and below bridge)
TNH-1717	treculii	GB x SP	0.62	0.37	0.00	0.01	Leon River at Taylors Valley Rd (~250m reach)
TNH-1723	treculii	LM	0.00	0.00	0.00	0.99	Leon River at Taylors Valley Rd (~250m reach)
TNH-1726	treculii	GB x SP	0.59	0.41	0.00	0.00	Leon River at Taylors Valley Rd (~250m reach)
TNH-1746	treculii	GB	0.98	0.02	0.00	0.00	Bear Creek on Collins Ranch
TNH-1764	treculii	GB x SP	0.69	0.31	0.00	0.00	San Gabriel River at CR100
TNH-1766	treculii	GB x SP	0.68	0.31	0.00	0.00	San Gabriel River at CR100
TNH-1769	treculii	GB x SP	0.65	0.34	0.00	0.00	San Gabriel River at CR100
TNH-1770	treculii	GB x SP	0.58	0.41	0.00	0.00	San Gabriel River at CR100
TNH-1773	treculii	GB x SM	0.92	0.00	0.08	0.01	San Gabriel River at CR100
TNH-1775	treculii	GB x SP x SM	0.62	0.28	0.10	0.01	San Gabriel River at CR100
TNH-1776	treculii	GB x SP	0.51	0.48	0.01	0.00	Little River at Sunshine Rd.
TNH-1777	treculii	GB x SM	0.82	0.02	0.16	0.01	Little River at Sunshine Rd.

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TNH-1778	treculii	GB x SP x SM	0.43	0.37	0.21	0.00	Little River at Sunshine Rd.
TNH-1779	treculii	GB x SP	0.66	0.34	0.00	0.00	Little River at Sunshine Rd.
TNH-1780	treculii	GB x SP	0.60	0.39	0.00	0.01	Little River at Sunshine Rd.
TNH-1781	treculii	GB x SP	0.51	0.48	0.01	0.01	Little River at Sunshine Rd.
TNH-1782	treculii	GB x SP x SM	0.09	0.67	0.24	0.01	Little River at Sunshine Rd.
TNH-1788	treculii	GB x SP	0.30	0.69	0.00	0.01	Lampasas River at FM1123
TNH-1791	treculii	GB x SP	0.66	0.34	0.00	0.00	Nolan Creek at E Ave A
TNH-1792	treculii	GB x SP	0.45	0.54	0.00	0.00	Nolan Creek at E Ave A
TNH-1802	treculii	GB x SP	0.77	0.22	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1803	treculii	GB x SP	0.69	0.31	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1804	treculii	GB x SP	0.69	0.31	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1805	treculii	GB x SP	0.48	0.52	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1806	treculii	GB x SP	0.69	0.31	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1807	treculii	GB x SP	0.68	0.31	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1808	treculii	GB x SP	0.52	0.47	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1809	treculii	GB x SP	0.58	0.41	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1810	treculii	GB x SP	0.58	0.42	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1811	treculii	GB x SP	0.66	0.34	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1812	treculii	GB x SP	0.52	0.47	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1813	treculii	GB x SP	0.52	0.47	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1814	treculii	GB x SP	0.52	0.47	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1815	treculii	GB x SP	0.68	0.31	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1816	treculii	GB x SP	0.52	0.47	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1821	treculii	GB x SP	0.78	0.21	0.00	0.01	Salado Creek at E. Amity Rd
TNH-1823	treculii	GB x SP	0.69	0.31	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1824	treculii	GB x SP	0.52	0.47	0.00	0.00	Salado Creek at E. Amity Rd
TNH-1830	treculii	GB	0.97	0.02	0.00	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1831	treculii	GB x SP	0.58	0.41	0.00	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1832	treculii	GB x SM	0.82	0.02	0.16	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1833	treculii	GB x SP	0.81	0.18	0.01	0.01	Lampasas River at SH195 (Ding Dong, TX)
TNH-1835	treculii	GB x SM	0.92	0.01	0.07	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1836	treculii	GB x SP	0.58	0.41	0.00	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1837	treculii	GB x SP	0.87	0.08	0.03	0.02	Lampasas River at SH195 (Ding Dong, TX)
TNH-1838	treculii	GB x SP	0.77	0.22	0.00	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1840	treculii	GB x SP	0.81	0.18	0.01	0.01	Lampasas River at SH195 (Ding Dong, TX)
TNH-1841	treculii	GB x SP	0.69	0.31	0.00	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1842	treculii	GB x SP	0.69	0.31	0.00	0.00	Lampasas River at SH195 (Ding Dong, TX)
TNH-1867	treculii	GB x SP	0.59	0.41	0.00	0.00	Sulphur Creek at FM1715
TNH-1868	treculii	GB x SP	0.69	0.31	0.00	0.00	Sulphur Creek at FM1715
TNH-1869	treculii	GB x SP	0.69	0.31	0.00	0.00	Sulphur Creek at FM1715
TNH-1870	treculii	GB x SM	0.83	0.01	0.17	0.00	Sulphur Creek at FM1715
TNH-1871	treculii	GB x SP	0.58	0.41	0.00	0.00	Sulphur Creek at FM1715
TNH-1872	treculii	GB x SP	0.69	0.31	0.00	0.00	Sulphur Creek at FM1715
TNH-1873	treculii	GB	0.98	0.02	0.00	0.00	Sulphur Creek at FM1715
TNH-1874	treculii	GB x SP	0.69	0.31	0.00	0.00	Sulphur Creek at FM1715
TNH-1875	treculii	GB	0.98	0.02	0.00	0.00	Sulphur Creek at FM1715
TNH-1876	treculii	GB	0.98	0.02	0.00	0.00	Sulphur Creek at FM1715
TNH-1877	treculii	GB x SP x SM	0.64	0.18	0.19	0.00	Sulphur Creek at FM1715
TNH-1950	treculii	GB x SP	0.79	0.20	0.00	0.01	Little River at US77 (S. of Cameron)
TNH-1951	treculii	GB	0.97	0.02	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1952	treculii	GB x SP	0.69	0.31	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1953	treculii	GB x SP	0.79	0.21	0.00	0.01	Little River at US77 (S. of Cameron)
TNH-1954	treculii	GB x SP	0.66	0.33	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1955	treculii	GB x SP	0.43	0.57	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1957	treculii	GB x SP	0.48	0.52	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1958	treculii	GB x SP	0.69	0.31	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1959	treculii	GB x SP	0.48	0.52	0.00	0.00	Little River at US77 (S. of Cameron)

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TNH-1960	treculii	GB x SP	0.59	0.41	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1962	treculii	GB x SP	0.58	0.41	0.00	0.00	Little River at US77 (S. of Cameron)
TNH-1963	treculii	GB x SP	0.61	0.37	0.01	0.02	Little River at US77 (S. of Cameron)
TNH-1964	treculii	GB x SP	0.79	0.20	0.00	0.01	San Gabriel River at FM487
TNH-1968	treculii	GB x SP	0.59	0.40	0.00	0.00	Brushy Creek at FM908
TNH-1976	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1977	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1980	treculii	GB x SP	0.92	0.04	0.01	0.03	Brushy Creek at Veterans Park
TNH-1982	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1983	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1984	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1985	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1986	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1987	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1989	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1992	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1994	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-1995	treculii	GB	1.00	0.00	0.00	0.00	Brushy Creek at Veterans Park
TNH-2001	treculii	GB x SP	0.83	0.17	0.00	0.00	Lake Creek at Lake Creek Park, Round Rock
TNH-2002	treculii	LM	0.00	0.00	0.00	1.00	Lake Creek at Lake Creek Park, Round Rock
TNH-2003	treculii	GB x SP	0.83	0.17	0.00	0.00	Lake Creek at Lake Creek Park, Round Rock
TNH-2004	treculii	LM	0.00	0.00	0.00	0.99	Lake Creek at Lake Creek Park, Round Rock